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Genomic Evidence of In-Flight SARS-CoV-2 Transmission, India to Australia, April 2021

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Epidemiologic and genomic investigation of SARS-CoV-2 infections associated with 2 repatriation flights from Australia to India in April 2021 indicated that 4 passengers transmitted SARS-CoV-2 to ≥11 other passengers. Results suggest transmission despite mandatory mask use and predeparture testing. For subsequent flights, predeparture quarantine and expanded predeparture testing were implemented.

During the first epidemic wave of SARS-CoV-2, Australia closed its borders; during March 28, 2020–November 1, 2021, international arriving passengers were required to undergo mandatory supervised quarantine (*1*). This initial response contributed to the end of the first pandemic wave in June 2020 and resulted in periods of COVID-19 control throughout the country (*2*).

Beginning October 23, 2020, a quarantine facility in Darwin, Northern Territory, Australia, received persons who arrived via government-assisted repatriation flights. On April 15 and 17, 2021, two repatriation flights (flights 1 and 2) carrying pas-

Table. Detailed information of case-patients belonging to SARS-CoV-2 genomic clusters detected after 2 flights from India to Darwin, Northern Territory, Australia, on April 15 and April 17, 2021*

Cluster and case-patient	Age group, y/sex	Family group	Virus Pango lineage	Cycle threshold	Symptom onset date	Date tested positive	Vaccinated	Seat no.
1								
A	30–39/M	None	B.1.617.2	14.3	Asymptomatic	Apr 15	N	56B
B	40–49/M	I	B.1.617.2	15.6	Asymptomatic	Apr 15	N	43D
C	20–29/F	I	B.1.617.2	11.6	Apr 20	Apr 20	N	43E
D	1–5/F	I	B.1.617.2	11.6	Asymptomatic	Apr 20	N	43F
E	<1/M	I	B.1.617.2	12.2	Asymptomatic	Apr 20	N	43D
F	30–39/M	II	B.1.617.2	22.6	Apr 20	Apr 20	N	43K
G	10–19/F	II	B.1.617.2	18	Apr 20	Apr 20	N	43H
H	1–5/M	II	B.1.617.2	26.5	Asymptomatic	Apr 20	N	43J
I	<1/F	II	B.1.617.2	19	Asymptomatic	Apr 20	N	43K
2								
J	20–29/F	None	B.1.617.1	12.4	Apr 16	Apr 15	N	42A
K	50–59/M	None	B.1.617.1	16.6	Apr 17	Apr 20	N	51H
L	1–5/M	III	B.1.617.1	22	Asymptomatic	Apr 22	N	42B
M	1–5/M	III	B.1.617.1	18.1	Apr 22	Apr 22	N	43B
N	30–39/F	III	B.1.617.1	20	Asymptomatic	Apr 22	N	43B
3								
O	50–59/F	IV	B.1.617.2	14.9	Asymptomatic	Apr 15	Y	3E
P	60–69/M	IV	B.1.617.2	14.9	Apr 15	Apr 16	Y	3F
Q	10–19/F	None	B.1.617.2	11.4	Asymptomatic	Apr 17	N	4E
4								
R	50–59/M	V	B.1.617.2	14.9	Asymptomatic	Apr 22	N	55A
S	60–69/F	V	B.1.617.2	14.9	Apr 22	Apr 23	N	55B
5								
T	10–19/M		B.1.617.2	11.7	Apr 17	Apr 18	N	48C
U	30–39/M	VI	B.1.617.2	16.1	Asymptomatic	Apr 24	N	48B
V	30–39/F	VI	B.1.617.2	12.8	Not available	Apr 24	N	48A
W	1–5/M		B.1.617.2	24.5	Asymptomatic	Apr 24	N	48J
6								
X	30–39/M	VII	B.1.1.7	10.9	Asymptomatic	Apr 17	N	43F
Y	40–49/M	VII	B.1.1.7	13.1	Asymptomatic	Apr 17	N	43E

sengers from 2 regions of India experiencing major COVID-19 outbreaks landed in Darwin. The percentages of passengers positive for COVID-19 were substantially greater for these 2 flights (24/164 [15%] and 23/181 [13%]) than for all previous repatriation flights to Darwin (225/9,651 [2%] during October 2020–April 2021).

In the 48 hours before flying, all passengers on the 2 flights had tested negative for SARS-CoV-2 by quantitative reverse transcription PCR (qRT-PCR). All passengers except infants and children were required to wear masks (*3*). COVID-19 vaccination coverage among passengers was low; 24/345 (7%) passengers had received ≥1 dose, and only 14 had received 2 doses of the same vaccine ≥14 days apart. At arrival, passengers entered quarantine, where they were tested for SARS-CoV-2 by qRT-PCR on days 0, 7, and 12, in addition to testing if symptomatic (Appendix 1, https://wwwnc.cdc.gov/EID/article/28/7/21-2466-App1.pdf).

Of the 47 passengers with positive results, 21 tested positive at arrival (arrival case-patients) and 26 tested positive ≥1 day after arriving in quarantine (quarantine case-patients) (Appendix 1 Figures 1, 2). Of the 21 arrival case-patients (Table), 18 were asymptomatic. qRT-PCR cycle threshold values were available for 18/21 (86%) arrival case-patients; median was 15.2 (range 8.4–34.1) cycles. For quarantine case-patients, median time of symptom onset was 5 (range 0–8) days after arrival, and the median number of days from arrival to a positive test result was 4 (range 1–7) days.

Among 41 (87%) of 47 SARS-CoV-2 genome sequences generated from case-patients on flights and 1 and 2, variant types were Delta (B.1.617.2) for 27 (57%), Kappa (B.1.617.1) for 10 (21%), Alpha (B.1.1.7) for 3 (6%), and A.23.1 sublineage for 1 (2%). Of 41 sequences, 25 (59%) belonged to 1 of 6 genomic clusters (Table; Figure; Appendix 1 Figure 3).

To determine whether infections were likely to have been acquired during flight, we analyzed case interviews, flight manifests, and genomic sequencing. Of the 21 arrival case-patients, 4 (19%) (identified as B, J, O, and T) on both flights were likely to have transmitted SARS-CoV-2 to ≥11 other passengers (F–I, L–N, Q, and U–W) who had sequences that belonged to the same SARS-CoV-2 genomic clusters, who did not belong to the same family group of an arrival case-patient, and who had been seated within 2 rows of an arrival case-patient. Using this information, we calculated secondary attack rates of 6% (8/143) for flight 1 and 2% (3/168) for flight 2. Five case-patients (C–E, P, and Y) with genomically linked virus belonged to arrival case family groups for which transmission possibly occurred before, during, or after the flight. One case-patient (K) with virus belonging to a genomic cluster was seated >2 rows from an arrival case-patient with genomically linked virus. Virus from 2 quarantine case-patients (R and S) genomically linked them to each other but not to an arrival case-patient (Table; Figure; Appendix 1). Only 5 quarantine case-patients from the flights had sequences that did not belong to a SARS-CoV-2 genomic cluster (Appendix 1 Figures 1, 2). Genomics refuted transmission to 6 quarantine case-patients seated within 2 rows of an arrival case-patient, linking 3 to a different cluster.

Soon after the 2 repatriation flights reported here, other repatriation flights from India were suspended, but flights resumed on May 15, 2021, when mandatory 72-hour preflight quarantine of passengers within India was instituted and testing of passengers was expanded to include rapid antigen testing on entry to preflight quarantine, qRT-PCR testing 48 hours before departure, and rapid antigen testing on the day of departure (*4*). During May 15–October 14, 2021, SARS-CoV-2 test results were positive for 13 (0.29%) of 4,543 passengers on repatriation flights from India and 30 (0.28%) of 10,679 passengers on repatriation flights to Darwin. Probable contributors to reduced repatriation cases were increasing vaccination rates and abatement of the Delta wave in India and globally (*5*).

At the time of this study, COVID-19 vaccination rates in Australia were low, most jurisdictions had little or no community transmission of SARS-CoV-2, and quarantine was key to reducing international incursions. We could not exclude transmission in the departure lounge and during boarding; however, spatial proximity of case-patients who did not belong to the same family groups but had genomically linked virus supported in-flight transmission. Previous studies that reported in-flight transmission of SARS-CoV-2 (*6*–*10*) did not include preflight testing, whereas our study included complete preflight and postflight testing and genomic sequencing. In conclusion, our investigation revealed evidence of flight-associated SARS-CoV-2 transmission on 2 repatriation flights from India to Australia during the Delta variant wave in April 2021.

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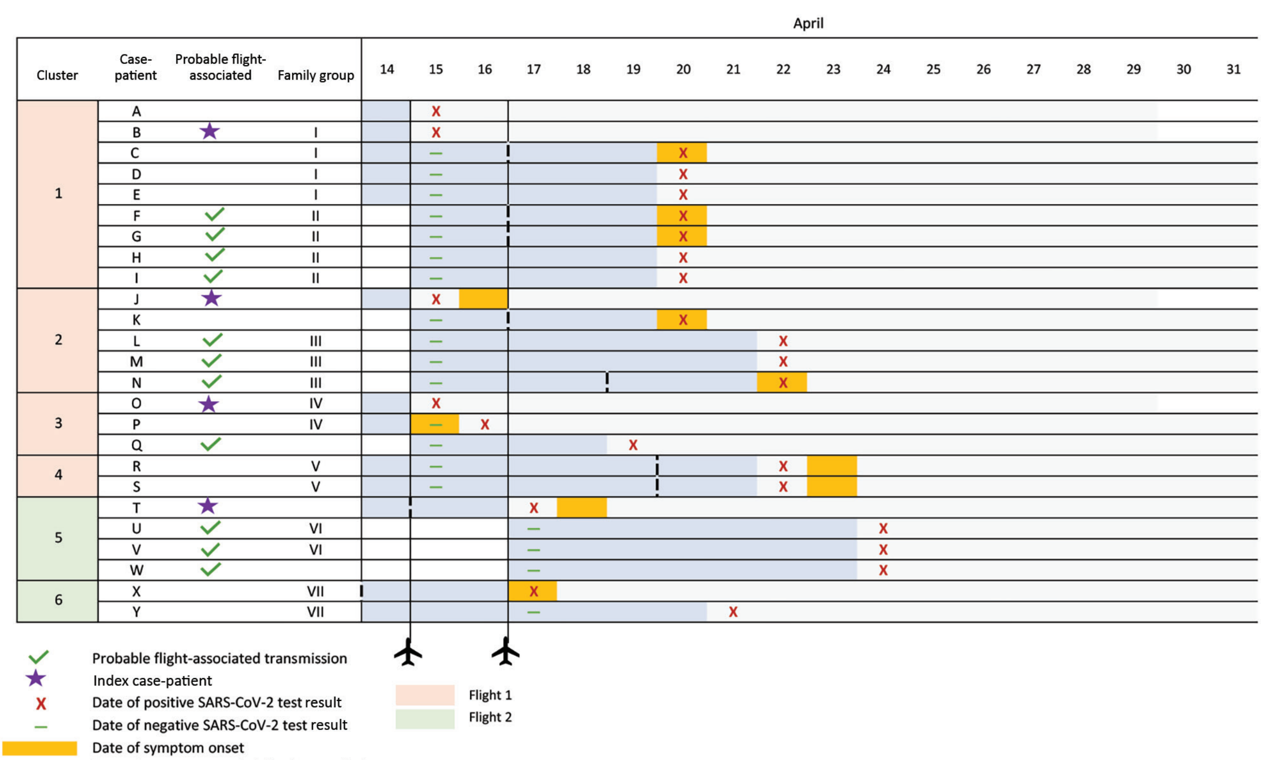

Figure. Schematic showing genomic clusters and in-flight transmission of SARS-CoV-2 on 2 flights from India to Australia, April 2021.

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Strongyloides Hyperinfection Syndrome among COVID-19 Patients Treated with Corticosteroids

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Widespread use of corticosteroids for COVID-19 treatment has led to *Strongyloides* reactivation and severe disease in patients from endemic areas. We describe a US patient with COVID-19 and *Strongyloides* hyperinfection syndrome and review other reported cases. Our findings highlight the need for *Strongyloides* screening and treatment in high-risk populations.

Strongyloidiasis is caused by the soil-transmitted helminth *Strongyloides stercoralis* and affects ≈613.8 million persons worldwide (*1*). *S. stercoralis* infections can be asymptomatic or chronic or can cause life-threatening larva dissemination, especially in immunocompromised patients (*2*).

Among COVID-19 patients, dexamethasone is the standard treatment for persons requiring supplemental oxygen, but among persons from *Strongyloides*-

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Genomic Evidence of In-Flight SARS-CoV-2 Transmission, India to Australia, April 2021

Appendix 1

Management of the flights

On arrival to Darwin, passengers from flight 1 and flight 2 arrived at a separate air terminal then travelled by bus by seating cohorts to the Howard Springs International Quarantine Facility (HSIQF). Passengers were seated at least 1.5 meters apart and masks were mandatory for persons ≥12 years old unless there was a medical contraindication (*1*). At the HSIQF passengers were physically separated into family groups in self-contained units that were well-ventilated with separate, non-communal bathroom facilities.

Individuals with a positive result on routine testing were moved to a separate zone within the facility for COVID-19 management. Close contacts of COVID-19 cases were moved to a second separate zone and required to quarantine for at least 14 days from their last exposure to the case and were monitored daily for fever and respiratory symptoms (*2*). On April 16, 2021, enhanced safety measures including the requirement for staff working with cases or close contacts to be fully vaccinated, wear full personal protective equipment, and undergo daily testing for SARS-CoV-2 by bilateral nasal swab rapid antigen testing in addition to daily saliva PCR confirmation. There were no instances of transmission between family groups or to staff within the HSIQF during this period.

SARS-CoV-2 testing, genomic sequencing, and bioinformatics analysis

Testing for SARS-CoV-2 was performed on swabs collected from the oropharynx and bilateral deep nasal passages, using the RT-qPCR assay from AusDiagnostics (Australia) with primers for the ORF1a and ORF8 genes. RT-qPCR cycle threshold (Ct) values reported in this manuscript are for the ORF8 target.

Genomic sequencing and consensus sequence generation were undertaken at the Microbiological Diagnostic Unit Public Health Laboratory at the Doherty Institute, Melbourne as described by Lane et al (*3*). In brief, tiled amplicons were generated using the ARTIC version 3 primers (https://github.com/artic-network/artic-ncov2019/tree/master/primer_schemes/nCoV-2019), libraries were prepared using NexteraXT, and sequencing was undertaken on the NextSeq500/550 or iSeq100 (Illumina) using 150bp paired-end reads. Reads were aligned to the Wuhan-Hu-1 reference (Genbank MN908947.3) to generate consensus sequences, which were uploaded to GISAID (https://www.gisaid.org; Appendix 2 Table 1, https://wwwnc.cdc.gov/EID/article/28/7/21-2466-App2.pdf). SARS-CoV-2 lineages were assigned using Pangolin v3.1.5 (*4*,*5*). Genomes belonging to lineages B.1.617.2 (Delta variant) and B.1.617.1 (Kappa variant) were included in a phylogenetic analysis with 300 publicly available SARS-CoV-2 genomes for context. To select context genomes, all 3,162 B.1.617 sequences from India between April 1, 2021, and April 15, 2021 with ≤5% missing or ambiguous base calls were downloaded from GISAID (https://www.gisaid.org) on September 13, 2021, and 300 of these were randomly selected for inclusion (Appendix 2 Table 2). Genomes were aligned to the Wuhan-Hu-1 reference genome using MAFFT v7.464.11 (*6*), and problematic sites (https://github.com/W-L/ProblematicSites_SARS-CoV2#human-friendly-version-of-the-vcf-file; last updated July 28, 2021) were masked from the alignment. Phylogenetic analysis was undertaken using IQ-TREE v1.6.12 (*7*) using a generalised time reversible model with 4 gamma categories and 1,000 ultrafast bootstrap replicates. The phylogenetic tree was annotated using the ggtree package in R v4.0.2. Genomic clusters from the flights were identified by visualisation of the phylogenetic tree.

Case definitions

Unless a negative RT-qPCR result indicated otherwise, we assumed the pre-symptomatic infectious period to be 1–3 days (*2*,*8*,*9*), and the incubation period to range from 1–14 days (*2*,) (C. Daley, unpub. data, https://www.medrxiv.org/content/10.1101/2020.12.23.20248790v1) *10–13*).

We defined arrival cases as passengers on flight 1 and flight 2 landing in Darwin on April 15 and 17 2021 respectively who tested positive to SARS-CoV-2 by RT-qPCR on day 0 of their quarantine period. Quarantine cases were defined as passengers who tested positive to SARS-CoV-2 by RT-qPCR ≥ 1 day after arriving in quarantine. Their infection was determined to result from probable flight-associated transmission if they were seated inside the 2x2 area of an arrival case (that is, within two rows either side of the arrival case); they were not a travel companion of an arrival case; they returned a SARS-CoV-2 virus genome sequence separated by ≤2 single nucleotide polymorphisms to that of an arrival case in the 2x2 area; and their SARS-CoV-2 genome was located on the same distal clade in the phylogenetic tree as the arrival case in the 2x2 area.

Detail of the clusters

Flight 1

Cluster 1

Cluster 1 included 9 cases with B.1.617.2 (Delta variant) infection (Figure 1; Figure 3). Two arrival cases (arrival cases A and B) belonging to this cluster were asymptomatic and had Ct values of 14.3 and 15.6 cycles. No plausible epidemiologic link prior to the flight was identified between the two arrival cases. Arrival case B, seated in row 43, was within the same 2x2 area as 7 individuals who were subsequently diagnosed with COVID-19 while in quarantine; these included 3 of their own family members (quarantine cases C, D, and E; family group I) and 4 members of a separate family (quarantine cases F, G, H, and I; family group II), all of whom were seated in row 43 and tested positive for SARS-CoV-2 on day 5 of quarantine. Members of family group I may have been infected before, during, or after the flight, while family group II who were travelling in the same row were attributed to probable flight-associated transmission.

Cluster 2

Cluster 2 included 5 cases with B.617.1 (Kappa variant) infection (Figure 1). One case (arrival case J) had a Ct value of 12.4 cycles and developed COVID-19 symptoms on day 1. This case was seated in row 42. Three members of a different family group (quarantine cases L, M, and N; all belonging to family group III) seated in row 43 subsequently tested positive to SARS-CoV-2 on the routine day 7 test. The infections of these three cases were determined to result from probable flight-associated transmission. A further quarantine case (quarantine case K) diagnosed on day 5 of quarantine was seated separately in row 51 and had no known epidemiologic links to the other cases in the cluster.

Cluster 3

Cluster 3 included 3 cases belonging to B.1.617.2 (Delta variant) (Figure 1; Figure 3). One case (arrival case O) seated in row 3 was asymptomatic and had a Ct value of 14.9 cycles. Their partner (quarantine case P; family group IV) had a negative arrival test but tested positive for SARS-CoV-2 on day 1 of quarantine. A third case (quarantine case Q) seated in row 4, not travelling with the other two cases, tested positive for SARS-CoV-2 on day 2. The infection of this case was determined to result from probable flight-associated transmission.

Cluster 4

Cluster 4 included 2 cases belonging to B.1.617.2 (Delta variant) (Figure 2; Figure 3). The two cases (quarantine case R and S) seated in row 55 belonged to the same family group (family group V) and both tested positive to SARS-CoV-2 on the routine day 7 test.

Flight 2

Cluster 5

Cluster 5 included four cases belonging to B.1.617.2 (Delta variant) (Figure 2; Figure 3). One case (arrival case T) seated in row 48 had a Ct value of 12.8 cycles and developed symptoms on day 1. Three further cases (quarantine cases U, V, and W) in row 48 belonging to two different travelling groups (including family group VI) tested positive for SARS-CoV-2 on routine day 7 testing. The infections of these three cases were determined to result from probable flight-associated transmission.

Cluster 6

Cluster 6 included two cases belonging to B.1.1.7 (Alpha variant) (Figure 2). One case (arrival case X) developed symptoms on day 0 and had a Ct value of 11.7 cycles (Figure 2). A member of their family (quarantine case Y; family group VII) subsequently tested positive for SARS-CoV-2 on day 4.

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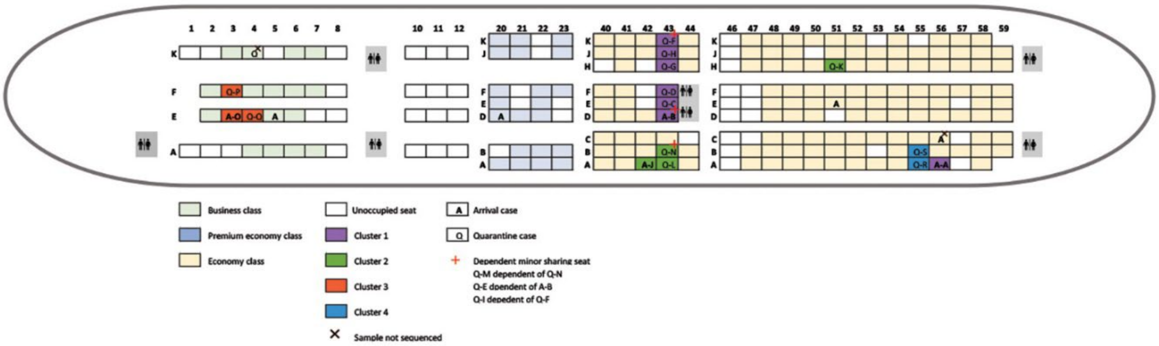
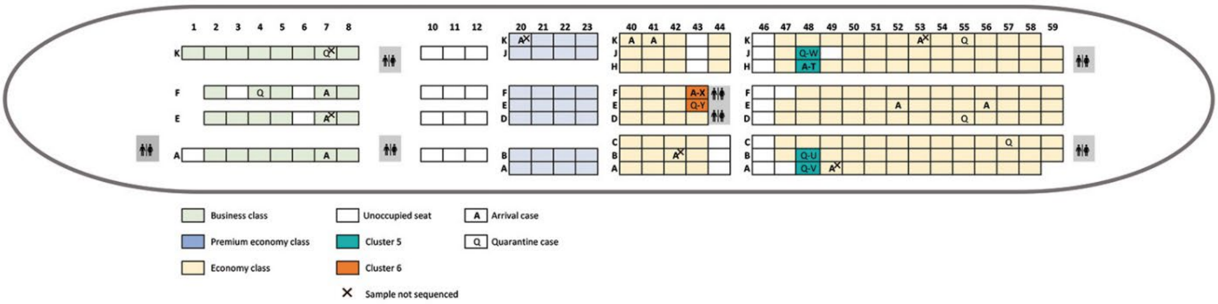
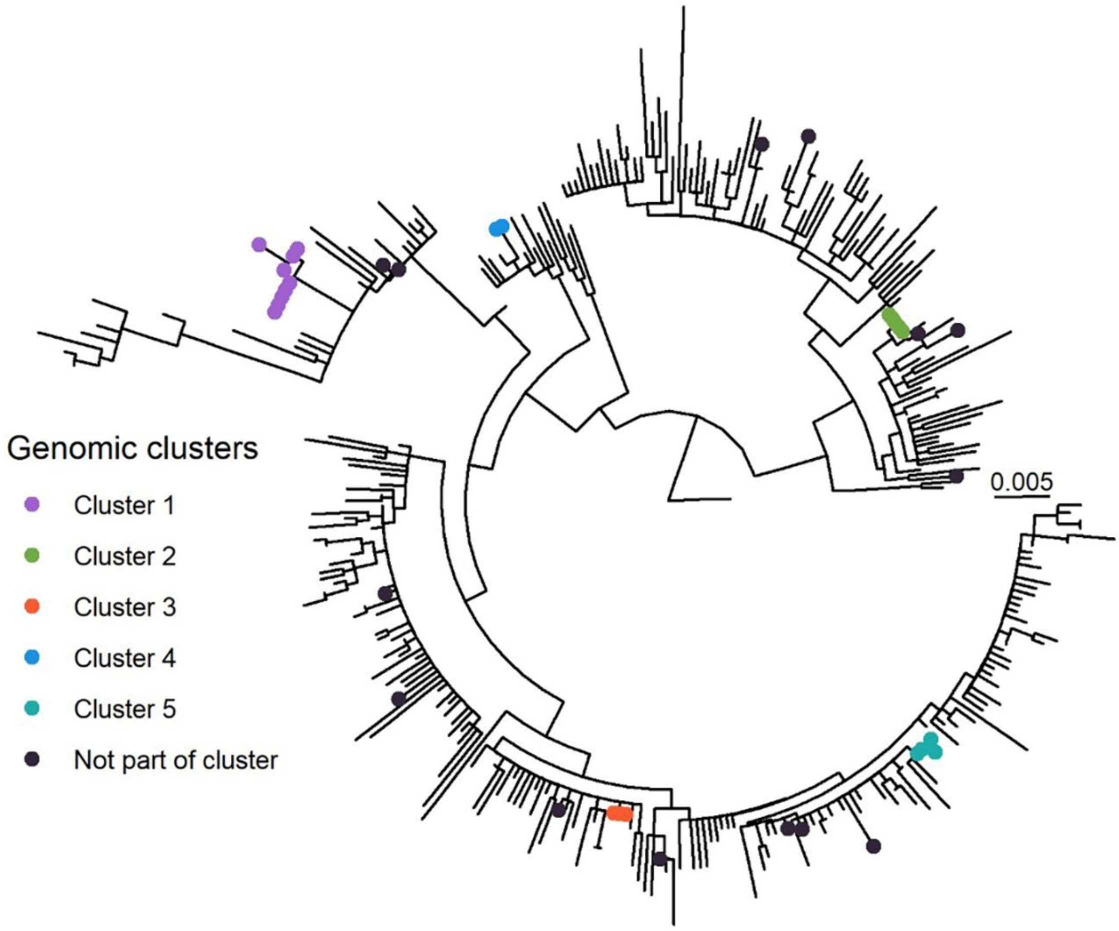


Appendix Figure 1. Seating plan and spatial distribution of arrival and quarantine cases of SARS-CoV-2 aboard flight 1 from India to Darwin, Australia, on April 15, 2021. Occupied seats are shaded. Note that quarantine cases E and I and (cluster 1) and quarantine cases N (cluster 2) were minors sharing a seat with an adult.



Appendix Figure 2. Seating plan and spatial distribution of arrival and quarantine cases of SARS-CoV-2 aboard flight 2 from India, to Darwin, Australia, on April 17, 2021. Occupied seats are shaded.



Appendix Figure 3. SARS-CoV-2 B.1.617 maximum likelihood phylogenetic tree including genomes from passengers with COVID-19 from flight 1 from India to Darwin, April 15, 2021, and from flight 2 from India to Darwin, April 17, 2021. The tree was rooted with the MN908947.3 reference genome, and the scale bar indicates substitutions/site.